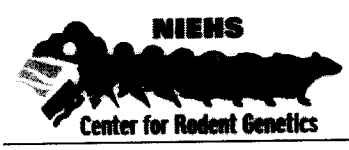

The

National Institute of Environmental Health Sciences

Coordinating Center for Rodent Genetics ("CRG")

Prepared by

William T Schrader, Ph.D.[1]

Deputy Scientific Director

NIEHS

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[1] Contact information: Tel. 919-541-3433; email schrader@niehs.nih.gov

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Introduction

Recent advances in molecular genetics and genomics, including the completion of The Human Genome Project, have changed the landscape of biological research and brought focus to the importance of human genetics in human biology. The role of genetic mutation in human heritable disease has been firmly established for many years. However, it is now apparent that genetic components influence susceptibility to and progression of many acute and chronic human diseases as well as normal human development, reproduction, aging and behavior. Some human diseases have both inherited and sporadic forms, indicating that a phenotype can correlate to multiple genotypes; this observation also reflects the fact that both germ line and somatic mutation play roles in human disease pathology. In addition, numerous biological characteristics and pathological states result from the coincident influence of several genetic factors, further increasing the complexity of the genetic component of biological characteristics. It has also recently been recognized that epigenetic factors play a significant role in many human diseases including cancer, adding still another layer of complexity to the genetic component of human biology.

In parallel with the revolution in molecular genetics and genomics, there is increasing awareness of the interconnectedness of human health and environmental health and of the fragility of the environment in which humans live. Human industrialized civilization is having a large impact on the health of the local and global biological and physical environment, and the reciprocal effect of the environment on human health is equally significant. Historically, the effort to understand the interplay between the environment and human health has been considered primarily the domain of toxicology. Toxicologists have relied heavily on relatively well-established approaches, especially long-term rodent bioassays that detect biological endpoints of late disease (i.e., tumor development) or acute clinical signs of toxicity. However, the field of toxicology has recently begun the process of reinventing itself, in light of the rapid state of technological and conceptual change in molecular biology and genomics. It is impossible to ignore the value and potential of targeted analysis of the genetic components of toxic responses. Such targeted research is possible, because of dramatic advances in the ability to analyze and manipulate the genomes of animal model systems, including rodent model systems, and to correlate results in these animal models to humans. Thus, the new fields of toxicogenomics and pharmacogenomics have recently emerged to exploit these new research opportunities. As a result, significant advances are being made in understanding the genetic basis of disease susceptibility and drug efficacy.

Rodent models (and cell lines derived from them) are essential tools in biomedical research because they allow researchers to systematically ask and answer questions about a defined biological system. Rodent-derived studies are especially useful and informative in toxicology and pharmacology research, because one can readily manipulate the timing and magnitude of exposure to a specific agent and measure the dose-response relationship using quantitative methods. This approach lies at the heart of both traditional toxicology testing to identify human health hazards and compound evaluation for discovery of pharmaceuticals. In addition, animals at various stages of an exposure-disease continuum can be used in biomarker development. In this way, the

stages of response including early pathobiology, pre-clinical disease, and clinical disease states can be assessed individually for stage-specific molecular indicators. Such indicators can provide important leads for development of biomarkers for human disease.

Since the late 1980s when the first "designer mice" were developed for studying cancer susceptibility, researchers have been engineering mouse strains for specific research purposes. Some strains, such as Oncomouse™ strains, are considered general research tools. Many of these strains are commercially available and are now considered essential "reagents" in laboratories that use the mouse as an experimental model. Other strains are excellent models for specific human diseases including diabetes, obesity, Parkinson's disease, xeroderma pigmentosum, severe combine immunodeficiency disease and others, or are targeted disruptions of specific genes.

Purpose

The purpose of this document is to bring awareness to the importance of rodent models in fulfilling the promise of modern toxicogenetic and pharmacogenetic research in keeping with the NIEHS Mission. When this awareness is coupled with a desire to promote rapid progress in understanding, preventing and treating human disease and a recognition of the importance of efficient use of valuable research funding and other resources, the need for a mechanism to coordinate and promote leading edge research in rodent genetics and toxicogenetics seems imperative. Such an interdisciplinary program will have several essential components, an important one being the collection, maintenance and dissemination of engineered mouse models for disease susceptibility and modern toxicology research. This program will also create and maintain a database on rodent toxicogenetics, provide core services to the research community including essential genetic characterization, phenotyping and toxicology assays, and promote rodent toxicogenetics research and training through the NIEHS extramural program. Therefore, it is timely to establish a structure to coordinate these activities across all of the NIEHS enterprise. Because this endeavor will be relevant and valuable to scientists in many sectors of the biomedical community, the program is expected to evolve as an initiative tightly linked to other interested NIH Institutes or organizations. Because the program is envisioned primarily as an umbrella structure to facilitate productive use of resources, the name for the program is the Coordinating Center for Rodent Genetics (CRG).

Background

The mouse is a major research tool for the study of human disease and biologic mechanisms for several reasons. The genomes of mice, humans and other placental mammals are highly conserved, such that almost all human genes have counterparts in the mouse genome recognizable by cross-species hybridization. The haploid size of the mammalian genome (≈3 billion base pairs) and its underlying genomic organization has remained relatively constant. Thus, the cloning of a human gene often leads directly to the cloning of a mouse homolog, or the reverse. The mouse is the most developed mammalian model system offering highly developed traditional genetic techniques, a rich pathology database, excellent animal husbandry techniques, molecular genetic techniques including the ability to construct specific gene-altered strains by gene

targeting and a complete reference genomic sequence. Other advantages of the mouse as a representative mammal include its small size, short generation time and high fertility rate under laboratory conditions.

Mouse and rat rodent models are used in virtually all the sciences upon which U.S. environmental regulatory policy is based. The National Toxicology Program (NTP), the Food and Drug Administration and the pharmaceutical industry are all heavily invested in rodent model systems in part because of the massive amount of historical toxicology and efficacy studies that have employed these model systems.

A highly developed infrastructure to support research using rodent models is needed to optimize progress in toxicology and other fields of biomedical research. Furthermore, such an infrastructure will conserve research resources and promote efficiency by 1) disseminating useful and novel research tools and information, 2) facilitating collaborative efforts and 3) reducing duplication of effort. It should be emphasized that it requires sizeable resources to generate mice strains and maintain large healthy rodent colonies. Because it is so costly to establish and maintain the infrastructure for state-of-the-art rodent genetics research, even a modest increase in efficiency could have a large impact on bottom line costs and resource utilization.

Center (CRG) Program Components

Management Structure

The CRG is expected to require a small administrative/management component at NIEHS. This program will be directed from the Office of the Director, NIEHS, as it will involve both intramural and extramural components of NIEHS.

Repository/Database

The CRG will exist to improve the infrastructure at NIEHS and its sister Institutes for development and study of specific mouse lines that are useful in toxicology research. A main component of that infrastructure is a first class mouse repository/database that solicits, maintains and distributes relevant mouse strains including strains that are highly specialized for specific experimental purposes. All grantees and intramural researchers will utilize this repository, and the mouse models they generate will populate the repository/database.

Services Cores

Many state-of-the-art technologies are needed to characterize mouse models. It would therefore be highly appropriate, as suggested here, to centralize expertise and equipment for basic mouse strain characterization, because this effort will promote efficiency and lower costs. The CRG proposes to evaluate providing selected core services, such as: DNA/protein sequencing, global gene expression analysis (i.e., microarray, proteomics), pathology/histopathology, transgenic strain construction (knock-ins, knockouts) and imaging. Effort will be made to eliminate redundancy within NIEHS and other ICs for these services, and all NIEHS scientists working with rodent models will be encouraged to utilize the CRG or other NIH service cores.

Ongoing CRG Activities in Mouse Toxicogenetics

Resequencing Project

The laboratory mouse population includes more than 50 highly inbred laboratory strains and thousands of mutant mouse strains that have been selected for specific phenotypic traits. The inbred laboratory mouse strains each have a unique fixed genotype, so they can be manipulated in the laboratory as a group of homogeneous experimental subjects, with reproducible phenotypes and defined allelic composition. Despite the many advantages of the inbred mouse strains commonly used in laboratory research, the genetic factors that determine strain specific differences remain poorly characterized. In addition, genetic linkage studies in inbred mice can be difficult and it remains a tedious process to map genetic interactions in the mouse. Recognizing this fact, a recent discussion of the future of mouse genomics stated that adequate future progress requires "improved methods to map [and identify] mutant genes" and that "technology for genotyping SNPs may yield the necessary cost, scale and efficiency to map large numbers of mutant genes." [Science 291. 1253. (2001).]

The phenotypes of many inbred mouse strains are characterized by unique responses to environmental toxicants and pharmaceuticals. The molecular mechanisms for such strain specific properties are well understood only in a limited number of cases. Therefore, the laboratory strains are a rich source of experimental material for gene and pathway discovery, and valuable insight into toxicological mechanisms might be gained if these strains were systematically studied using genotype-phenotype association approaches, and if the genetic characterization of these strains were also accelerated.

Therefore, to maximize the usefulness of laboratory mouse strains and to stimulate use of the laboratory mouse in gene-environment interaction research, the NIEHS is establishing a mechanism to create a dense SNP map of the mouse genome by sequencing the non-repetitive portion of the genome in up to fifteen mouse strains chosen for their applicability to environmental health research and toxicology. Construction of this map will be initiated shortly, using high-throughput DNA sequencing. Based on preliminary sequencing experiments using genomic DNA from several mouse strains, it is estimated that the mouse SNP map will be remarkably dense, with approximately 1 polymorphic site per 150-400 bp. The CRG will coordinate this project with other related strain characterization activities and will ensure that the SNP data are widely available to mouse geneticists via the CRG database/website and other sources.

Extramural Research Activities

NIEHS currently supports over 250 extramural research initiatives that use or generate mouse models for mechanism-based environmental health research, studies of disease-associated susceptibility factors and toxicology. Two of the most important of these projects are (a) investigator-initiated research grants and (b) the Comparative Mouse Genomics Centers Consortium (CMGCC) (part of the Environmental Genome Project or "EGP"). The CMGCC has assembled a nationwide team of participating academic research centers, each headed by a prominent environmental scientist with specific research interests related to gene-environment interactions. Five centers are

currently operating under this arrangement. These activities already contribute to advances in mouse toxicogenetics research.

The main function of the CRG will be to stimulate additional research activity involving rodent models through various approaches. These approaches will be identified through a series of workshops that will include interactions with the Institute's National Advisory Council.

Institute-wide Training

NIEHS has been a leader in the use of rodent models in toxicology. Therefore, there is significant expertise in both mouse genetics and toxicology in the NIEHS portfolio at present. However, it will be important to encourage and provide incentives to scientists to enter this field. This is especially important given the rapid rate of change in the technologies that are available for toxicogenetic research. Thus, the CRG proposes to enhance training opportunities in toxicogenetics, whose purpose will be to recruit and retain outstanding students and professional scientists in this field of research. A small intramural project currently targets postdoctoral trainees who enter a laboratory setting with the dual objectives of carrying out hypothesis-driven bench research and evaluative studies of environmental agents via existing experimental methods. The expanded program will target entry-level toxicologists as well as senior investigators. Career advancement opportunities will also be promoted, including seminar programs, workshops, and an annual symposium at relevant national meetings.

Comparative Mouse Genomics Centers Consortium (CMGCC)

The goals of the Consortium are:

1) To identify relevant and feasible mouse models to be developed by the Consortium;

2) To identify mouse models that are relevant to human environmental health; and

3) To validate mouse models that are relevant to human environmentally-induced disease.

The Environmental Genome Project (EGP) was established by NIEHS to study how variation in human DNA sequences (genetic polymorphism) influences susceptibility to environmentally-induced disease. The knowledge developed by the EGP will be used to improve understanding of gene-environment interactions, and provide an enhanced science-based framework for environmental policy. The ultimate goal of the EGP, and of policies based on EGP research, is to prevent adverse effects from environmental exposure and to protect subset of individuals who are at higher risk for these adverse events.

The CMGCC was initiated by the EGP to develop transgenic and knockout mouse models based on human DNA sequence variants in environmentally responsive genes. These mouse models are tools to improve understanding of the biological significance of human DNA polymorphism. Initially, CMGCC is focusing on variation in genes involved in DNA repair or cell cycle control. These were chosen because many of them are well-characterized environmentally responsive genes and function in pathways that

have been validated by association with human disease phenotypes. Environmentally responsive genes also play roles in cell division, cell signaling, cell structure, gene expression, apoptosis, membrane channels, and metabolism.

Mutant mouse models generated by CMGCC to date include: CHK 2, Cyclin D1b, Rb, msh2, msh6 ,mlh1, pms2, pcna, pol gamma, eta, zeta, mu, Fen1, XPV, Cyclin D1, p21, E2F1, Brca1, PTEN, p53, Pol beta, Ercc1, Xpa, Ercc2 (XPD), XRCC1, ATM, MGMT, WRN, OGG1, APEX, p27, PRKR, Ligase 4.

Intramural Research Activities

NIEHS commercial contracts are already in place for generating selected mouse targeted genetic mutant strains as proposed by intramural scientists. These activities will be monitored within the coordinated CRG program as appropriate. Some activities under the topic of service cores (i.e., bioinformatics) may require additional contract or grant support.

Mouse models carrying an engineered or spontaneous alteration in a single gene are important tools for determining the role of a gene in a particular biochemical pathway. Gene deletions ("knock-out mice"), additions ("transgenic mice") and time- tissue- or sequence-specific alterations ("knock-in" or "conditional knockout" mice) are engineered using recombinant DNA technology, which allows virtually any mouse gene to be systematically altered. The recombinant mice carry the genetic mutation in all cells including germ line cells, enabling the defect to be transmitted to all progeny. The NIEHS intramural program has a contract mechanism in place for generating such transgenic/knockout mice on an as needed basis. The contract will generate up to 26 genetically engineered mouse lines per year.

When complete deficiency of a gene is lethal or when it interferes with normal development, a knockout model is not sufficient to analyze the function of that gene. In these cases, a conditional knockout has the potential to be much more informative. These animals are engineered to lose gene function in a tissue- or time targeted manner. NIEHS has identified approximately 40 mouse genes for which conditional knockout mouse lines will be especially valuable for ongoing research in environmental health issues.

National Toxicology Program Activities

The mission of the NTP is to coordinate toxicological evaluation programs within the Department of Health and Human Services (DHHS); strengthen the science base in toxicology; develop and validate improved testing methods; and provide information about potentially toxic chemicals to health regulatory and research agencies, the scientific and medical communities, and the public.

A number of testing modalities are used by the NTP, but an especially well-known one is the rat and mouse bioassay for carcinogenesis potential. Therefore, to fulfill its mission, the NTP has developed the capacity to produce and maintain uniform lines of mice. All mice used in NTP research must meet rigorous quality control standards. These mouse lines are monitored for the research needs of the NTP, including specifics of genotype/strain, size, weight, number, and gender. Animals are routinely tested for genetic homogeneity, microbial and parasitic status, viral and mycoplasma serology

profiles and pathological changes. Moreover novel mouse genetic mutant strains can be evaluated using the unique histopathology and compound susceptibility capabilities of the NTP for comparison of new models with earlier findings. This capacity is unique to NIEHS and can be leveraged to facilitate efforts of the CRG.

Strategic Plan Outline

Overlapping Phases in the NIEHS Coordinating Center for Rodent Genetics

Phase I: Investigational (Years 1-5)

- To achieve certain scientific milestones in three areas
  - Toxicogenetics and Pharmacogenetics
    - Environmental exposure and susceptibility
  - Molecular genetics
    - Mechanisms and pathways
  - Comparative genetics
    - Mouse & human biomarkers, predictive cell lines

Phase II: Translational Component Added (Years 3-8)

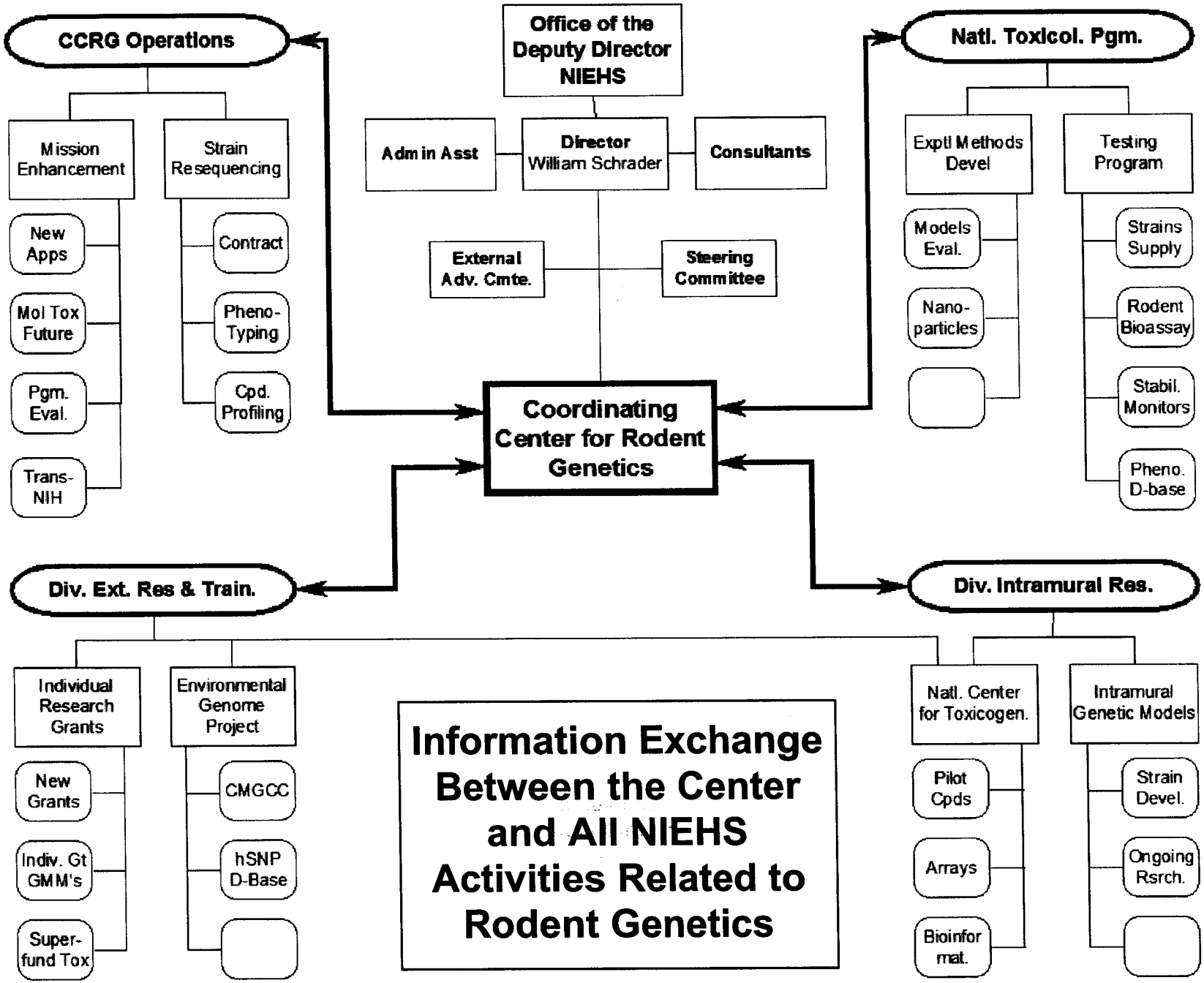
- Epidemiology, Population Analysis, NTP tests

Phase III: Interventional Component Added (Years 6-10)

- Therapeutic, Regulatory guidance

Summary

Rodents are major animal models for the study of human disease. Mouse models have proven especially useful for studying gene-environment interactions, have allowed us to understand risk factors for environmentally-associated disease and have provided opportunities to develop biomarkers of disease pathobiology and environmental exposure. It is well recognized that mouse and rat models are critical experimental tools for fulfilling the promise of modern toxicogenetic and pharmacogenetic research. Therefore, a mechanism to promote and coordinate use of rodent models in toxicology and other biomedical research will be invaluable to the entire scientific community. The NIEHS has therefore established the Center for Rodent Genetics (CRG), as an institutional and national resource that will stimulate research in rodent toxicogenetics and related fields. The CRG will foster state-of-the-art mouse repositories/databases, provide service cores to the research community, promote communication regarding mouse toxicogenetic research, and foster investigator-initiated research and training opportunities within the extramural scientific community. The existing infrastructure at NIEHS is conducive to establishing and managing the CRG .


CCRG Operations
Mission Enhancement
Strain Resequencing
New Apps
Mol Tox Future
Pgm. Eval.
Trans-NIH
Contract
Pheno-Typing
Cpd. Profiling
Office of the Deputy Director NIEHS
Admin Asst
Director William Schrader
Consultants
External Adv. Cmte.
Steering Committee
Coordinating Center for Rodent Genetics
Natl. Toxicol. Pgm.
Exptl Methods Devel
Testing Program
Models Eval.
Nano-particles
Strains Supply
Rodent Bioassay
Stabil. Monitors
Pheno. D-base
Div. Ext. Res & Train.
Individual Research Grants
Environmental Genome Project
New Grants
Indiv. Gt GMM's
Super-fund Tox
CMGCC
hSNP D-Base
Div. Intramural Res.
Natl. Center for Toxicogen.
Intramural Genetic Models
Pilot Cpds
Arrays
Bioinfor mat.
Strain Devel.
Ongoing Rsrch.
Information Exchange Between the Center and All NIEHS Activities Related to Rodent Genetics